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Relative testis size and sperm morphometry across mammals: no evidence for an association between sperm competition and sperm length

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Understanding why there is extensive variation in sperm form and function across taxa has been a challenge because sperm are specialized cells operating at a microscopic level in a complex environment. This comparative study collates published data to determine whether the evolution of sperm morphometry (sperm total length and separate component dimensions) is associated with sperm competition (when different males' sperm mix and compete for a female's ova) across 83 mammalian species. We use relative testes mass as an indicator of the intensity of sperm competition across taxa: relative investment into testes is widely accepted to predict the level of sperm competition that a species or population endures. Although we found evidence for positive associations between relative testes mass (controlling for allometry) and sperm morphometry across 83 mammalian species, these relationships were phylogenetically dependent. When we appropriately controlled for phylogenetic association using multiple regression within a phylogenetic framework, there was no relationship between relative testes mass and sperm length across mammals. Furthermore, we found no evidence for associations between relative testes mass and sperm head, mid-piece or flagellar lengths, nor was there a relationship with mid-piece or mitochondrial volumes. Results, therefore, indicate that sperm competition does not select for longer or shorter sperm across mammals, and alternative forces selecting on sperm form and function are discussed.

Keywords: mating pattern; phylogeny; allometry; gamete

1. INTRODUCTION

Sperm competition, when gametes from different males compete for fertilization, is recognized as a potent evolutionary force (Parker 1970; Smith 1984; Birkhead & Møller 1992, 1998) that has led to a rich array of sexually selected adaptations (Darwin 1871). These adaptations either enhance a male's own sperm competitiveness, or negate or eliminate rival sperm fitness, and range from the whole organism to the gamete (Birkhead & Møller 1998). Examples of such adaptations broadly range from behaviours such as mate guarding (Parker 1970) to sexually selected sperm architectures that are optimized for winning fertilizations before rival gametes (Radwan 1996; LaMunyon & Ward 1998).

One male reproductive trait that shows almost universally consistent adaptation to level of sperm competition is the relative size of the testes. This adaptation was first identified across primates (Short 1979) and has since been documented in a wide range of vertebrate and invertebrate taxa (Harcourt *et al.* (1981; primates); Kenagy & Trombulak (1986; mammals); Møller (1988*a*; primates); Møller (1988*b*), Møller & Briskie (1995; birds); Ginsberg & Rubenstein (1990; ungulates); Jennions & Passmore (1993; frogs); Gage (1994; butterflies); Hosken (1997, 1998; microchiropteran and megachiropteran bats) and Stockley *et al.* (1997; fishes)). In a review, Parker *et al.* (1997)

found that 12 out of 14 studies demonstrated significant positive effects of mating pattern and sperm competition risk on relative testis size, and only one study did not show a significant effect (Heske & Ostfeld 1990). There is therefore strong evidence that taxa whose mating pattern generates higher levels of sperm competition have evolved relatively larger testes than monogamous relatives, leading Short (1979) to describe testes as 'infallible predictors of the mating system' (p. 148).

These across-species testis size relationships are further supported by studies within species that show variation in testis size across populations in which males have evolved alternative mating strategies that generate different risks of sperm competition (Petersen & Warner 1998). In Atlantic salmon (*Salmo salar*), for example, large anadromous males attempt to defend their females from competition while the tiny precocious parr must sneak for fertilizations and always spawn under full competition (Jones 1959). Relative investment in testis size of parr is double that of anadromous males (Gage *et al.* 1995) and parr achieve a disproportionately high level of fertilization success for their body size (Hutchings & Myers 1988). Ultimately, males may evolve developmental plasticity that enables them to partition investment between somatic and gonadal tissue. In the meal moth *Plodia interpunctella*, testis size is dependent upon the risk of sperm competition into which a male is likely to emerge as an adult: males develop larger testes and produce bigger ejaculates when they have been reared in a high population density environment that will generate high sperm competition risks for reproducing

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adults (Gage 1995). Selection experiments on dung flies (*Scathophaga stercoraria*) provide clear empirical support for a direct relationship between sperm competition level and testis size (Hosken & Ward 2001): populations maintained under elevated sperm competition intensities were selected to develop larger testes than monogamous lines. There is therefore widespread and consistent evidence that sperm competition selects for males to partition investment towards gonadal tissue. Bigger testes enable males to produce more spermatozoa (Møller 1988*b*; Gage 1995) and, although sperm competition success is influenced by a range of spatial and/or temporal factors arising from males and/or females (Birkhead & Møller 1998), greater numbers of sperm enable males, fundamentally, to win more fertilizations (Martin *et al.* 1974; Parker 1982; Simmons 1987).

Increased sperm numbers are therefore an important general adaptive response to sperm competition, but is the wide variation recognized in sperm size also a result of sperm competition? Sperm show wide variation in size, from tiny 28 μm gametes of the porcupine *Hystrix africaeaustralis* (Gage 1998) to 58 290 μm giants in *Drosophila bifurca* (Pitnick *et al.* 1995). Sperm form is likely to dictate function and there is variation in sperm length and the dimensions of the midpiece and flagellum both across taxa (e.g. Gage 1998; Morrow & Gage 2000) and between individuals within species (Ward 1998; Morrow & Gage 2001). High sperm motility is essential for fertilization (Katz *et al.* 1989) and sperm competition success (Birkhead *et al.* 1999). Longer flagella may enable greater velocities and thrusting forces to be achieved (Katz & Drobnis 1990; Gomendio & Roldan 1991). It has been predicted that longer sperm may swim faster (Gomendio & Roldan 1993), but that the increased flagellar demands on mitochondria in the mid-piece may curtail sperm longevity (Stockley *et al.* 1997; Levitan 2000).

Several comparative studies have attempted to explain the variation across species in sperm length. Variance in sperm size is positively associated with dimensions of the female reproductive tract across beetles (Dybas & Dybas 1981), birds (Briskie & Montgomerie 1992), drosophilids (Pitnick *et al.* 1995), diopsid flies (Presgraves *et al.* 1999) and moths (Morrow & Gage 2000), indicating coevolution between sperm form and their functioning environment. Sperm competition also influences sperm size: across butterflies (Gage 1994), moths (Morrow & Gage 2000), cichlid fishes (Balshine *et al.* 2001) and birds (Briskie & Montgomerie 1992; Johnson & Briskie 1999), sperm size is positively associated with sperm competition risk. Across mammals, variable results have been found. Mammalian sperm are characteristically tiny, but vary more than 12-fold in length from 28 μm in the porcupine *Hystrix africaeaustralis* to 349 μm in the honey possum *Tarsipes rostratus* (Gage 1998), providing rich potential for exploring this variation. Gomendio & Roldan (1991) found that sperm size was greater in primate and rodent taxa that generated higher risks of sperm competition. By contrast, Harcourt (1991) and Dixson (1998) found no evidence for a relationship between sperm size and mating pattern across primates, although Dixson (1993) found some evidence for a positive relationship between relative testis size and sperm length across 15 primate species but suggested caution interpreting these results and did not control for

phylogenetic association. Most recently, Anderson & Dixson (2002) refined their analyses to show that mid-piece volume (but not sperm head or flagellum volume) was positively associated with sperm competition level across primates.

In this study, we explore the evolution of sperm length across mammals (analyses are conducted on data from 83 species in 12 orders containing 26 families) in relation to level of sperm competition using contemporary comparative analysis techniques (Felsenstein 1985; Harvey & Pagel 1991). We measured the strength of sperm competition by testis size adjusted for body size, which provides the least confounded and most consistent quantification of the level of sperm competition across mammalian taxa (Parker *et al.* 1997; Short 1997). By contrast, designations of mating pattern require detailed behavioural and/or paternity information to be consistently reliable across species, and are thus not generally available.

2. MATERIAL AND METHODS

(a) *Data collation*

Data on sperm dimensions, testis and body mass (see table 1) were collated from published sources and logarithmically transformed prior to comparative analysis (Harvey 1982). Sperm morphometry data were derived from references cited in Gage (1998) and that article's electronic appendix (http://www.pubs.royalsoc.ac.uk/publish/pro_bs/jan98pb2.htm). Sperm morphometry data included measures of sperm total length and the length of the sperm head, mid-piece and flagellum. Sperm size shows no allometry in mammals (Gage 1998).

Testes mass data were collated from Harcourt (1991; primates), Kenagy & Trombulak (1986; mammals), Ginsberg & Rubenstein (1990; ungulates), and Hosken (1997, 1998; bats). Data were derived from males in breeding condition. Testis mass scales allometrically with body mass across taxa and we therefore control for this association by calculating residuals (from the logarithmically transformed trait values) that are free of allometric association. To control for potential variance in body and testis mass between different populations of the same species, we always regressed body mass values on testis mass using those data that were presented within the same study.

(b) *Comparative analyses*

The data were analysed using a multiple regression analysis, conducted within a phylogenetic framework. The model is outlined below within a maximum-likelihood framework, but is exactly equivalent to a phylogenetic generalized least-squares analysis (e.g. Martins & Hansen 1997; Pagel 1997, 1999). The regression model is based on a general linear model as follows:

$$Y = Xb + e. \tag{2.1}$$

The n observations on the dependent variable y are expressed as a $1 \times n$ vector. The $k \times n$ matrix X is termed the design matrix. This matrix, as its name implies, defines the model to be fitted in terms of the $k - 1$ independent variables included, together with the intercept. All elements of the first column of X are set to unity. This column effectively acts as a dummy variable and codes for the model intercept. In the case of the full model, where both body size and testes size are included, X contains two further columns (i.e. $k = 3$), one listing the n measurements for each of the two independent variables. We then compared the fit of this model with reduced models in which only one or

Table 1. Analysis of sperm morphometry using multiple regression analysis.

((*a*) Analysis ignoring phylogenetic relationships between species. (*b*) Regression analysis controlling for phylogeny (see § 2*b* for details). The table shows the fitted models predicting total sperm length or the length of the three spermatozoal components as a function of body size and testes size. The coefficient (slope) for each of these is shown, together with χ^2 from log-likelihood ratio tests of whether these values are statistically significantly different from zero. The proportion of variance explained by the fitted model is also shown. In the case of the phylogenetic analysis in (*b*), an index of phylogenetic dependence, λ was also estimated. The χ^2 value for this parameter tests whether it is significantly different from zero, and hence whether the phylogenetic analysis is to be preferred. Note that in all cases the maximum-likelihood value of λ is significantly different from zero, and therefore the analysis in (*b*) is to be preferred over the non-phylogenetic analysis in (*a*).)

component	intercept	body size	χ^2	testes size	χ^2	λ	χ^2	r^2
(*a*)								
total length	4.366	−0.132	23.782 ($p < 0.0001$)	0.1087	10.182 ($p < 0.002$)			0.382
head	0.660	−0.088	7.562 ($p < 0.01$)	0.1155	8.354 ($p < 0.005$)			0.111
mid piece	1.100	−0.212	17.620 ($p < 0.0001$)	0.1865	9.386 ($p < 0.003$)			0.309
flagellum	1.769	−0.131	17.420 ($p < 0.0001$)	0.105	7.760 ($p < 0.01$)			0.351
(*b*)								
total length	4.42	−0.062	5.32 ($p = 0.021$)	0.042	1.76 (n.s.)	1.00	20.53 ($p < 0.0001$)	0.074
head	0.72	−0.073	3.32 (n.s.)	0.093	5.36 ($p = 0.021$)	1.00	6.66 ($p < 0.0001$)	0.075
mid piece	1.13	−0.091	4.10 ($p < 0.043$)	0.074	2.70 (n.s.)	1.00	29.79 ($p < 0.0001$)	0.058
flagellum	1.57	−0.041	1.82 (n.s.)	0.050	2.84 (n.s.)	1.00	20.53 ($p < 0.0001$)	0.041

the other of the two independent variables was included (i.e. $k = 2$). In this version of the model, X contains only one column listing the included variable in addition to the dummy variable. The vector b is a $1 \times k$ list of the model parameters, i.e. the intercept together with the slopes for each of the $k - 1$ independent variables in the model.

Assuming that the errors in equation (2.1) are normally distributed, the log likelihood of the data for a given vector of parameters b is

$$\log L = -\frac{1}{2}(\ln(2\pi) + n \ln\sigma + \ln|V| + (y - Xb)^{\mathrm{T}}(\sigma^2 V)^{-1}$$
$$(y - Xb)). \tag{2.2}$$

Equation (2.2) is the log likelihood for a normal distribution of correlated observations. In equation (2.3), correlations arise as a consequence of shared common ancestry between species. The $n \times n$ matrix V consists of diagonal elements that measure the expected variance of species' traits, while the off-diagonal elements measure the degree to which species' values are expected to vary as a consequence of common ancestry. In the case of phylogenetically independent evolution, all of the off-diagonal elements of V would be set to zero. In the case of correlated evolution, a Brownian model is commonly assumed (Felsenstein 1973; Pagel 1997, 1999) and the elements of V are given by the shared evolutionary paths of species. Thus, if two species shared a common ancestor from time zero to time t following which time they have undergone independent evolution, the expected covariance of traits between these species is proportional to t (Felsenstein 1973; Hansen & Martins 1996). The constant of proportionality is the time-scaled variance.

Equation (2.2) is readily solved to yield the maximum-

likelihood estimate of b through solving the well-known normal equations

$$b = (X^{\mathrm{T}}V^{-1}X)^{-1}(X^{\mathrm{T}}V^{-1}Y). \tag{2.3}$$

The vector of standard errors of the estimated parameters is given by the roots of the elements of the main diagonal of the parameter variance–covariance matrix (e.g. Dobson 1990):

$$s = \sigma^2(X^{\mathrm{T}}V^{-1}X)^{-1}. \tag{2.4}$$

The elements of s can then be used to perform significance tests on the estimated parameters.

This analysis is preferable to the more familiar method of independent contrasts, as this form of modelling allows the effects of each of the two variables to be estimated while statistically eliminating the effects of the other, as in a conventional multiple regression (Freckleton 2002). In particular the effects of testes size can be estimated while controlling for the effects of body size. It is important to ensure that data are appropriately transformed prior to phylogenetic analysis (Freckleton 2000), and preliminary analysis using Box–Cox transformations indicated that all variables should be logarithmically transformed.

(c) Testing for phylogenetic dependence

The results of phylogenetic and non-phylogenetic analyses frequently conflict. It is thus important to determine whether traits show evidence of phylogenetic dependence. To do this, we estimated an index of phylogenetic dependence. The index (λ) that we applied is a measure of the degree to which traits covary among species, relative to the constant variance Brownian model of evolutionary relationships that we assumed above (Pagel 1999; Freckleton *et al.* 2002). In this model it is assumed that

the covariance of traits between species is linearly related to their shared evolutionary paths. This contrasts with phylogenetically independent evolution, in which the covariance of traits among species would be independent of the amount of shared evolutionary history. In the case of phylogenetically independent evolution, the matrix V is a diagonal matrix, i.e. all of the off-diagonal elements are set to zero. The index λ is a multiplier of the off-diagonal elements of V that best fits the data. Thus, if the data show little phylogenetic structure, λ is estimated to have a low or zero value, whereas if the data are structured according to the Brownian model it will take a value of 1. The maximum-likelihood value of λ is found by a simple one-dimensional parameter search. The maximum-likelihood value of λ can be used to test whether data exhibit significant phylogenetic dependence using a likelihood ratio test. If $L(\lambda_{\mathrm{ML}})$ is the log likelihood of the model with λ set to its maximum-likelihood value, and $L(\lambda_{\mathrm{O}})$ is the log likelihood of the model with λ set to zero, then the log-likelihood ratio statistic is $-2(L(\lambda_{\mathrm{ML}}) - L(\lambda_{\mathrm{O}}))$, which should be χ^2-distributed with one degree of freedom under the null hypothesis that the inclusion of λ does not significantly improve the fit of the model.

3. RESULTS

The composite phylogeny for the species used in this study is shown in figure 1. Table 1 summarizes the results of the regression analysis. When species trait values were used as independent data points in regressions there were significant negative associations between total sperm length and body size, as well as significant negative associations between the size of sperm components and body size (table 1*a*). These regressions control for the effect of body size when estimating the effect of testes size, and reveal a significant positive relationship between testes size and total sperm length, as well as significant positive associations between the size of the various sperm components (table 1*a*). These species-level analyses thus indicate that the level of sperm competition (as predicted by testes size controlling for body mass) is positively associated with sperm length, and also with the three spermatozoal components: head, mid-piece and flagellum.

Phylogenetic analysis, however, fails to support these results (table 1*b*). There was no significant relationship between testes size and sperm length, or between testes size and the size of the mid-piece or flagellum. There was a statistically significant relationship between the size of the sperm head and testes size, although this relationship was extremely weak ($r^2 = 0.075$). The index of phylogenetic dependence, λ, indicated that the data exhibit strong phylogenetic dependence (table 1*b*), therefore the phylogenetic analysis was statistically better supported by the data. Therefore, based on these analyses that control for phylogenetic dependence, there is no evidence for relationships between testes size and sperm size. This is clear in figure 2, where sperm length is plotted against testes size. In figure 1, sperm length has been corrected for variations in body size between species using the regression from the phylogenetic analysis. Thus, figure 1 shows the effect of testes size independent of body size, and testes size should be an index of sperm competition in this plot. There is therefore no evidence that sperm competition affects sperm size in mammals.

4. DISCUSSION

This comparative study finds that the dimensions of mammalian spermatozoa show no association with the relative testis size of mammalian taxa, once phylogenetic associations are controlled for. Relative testis size is an indirect though reliable measure of the risk of sperm competition across related taxa, and accordingly the analyses across 83 mammalian species lend no support for an influence of sperm competition on sperm length. Our results contrast with Gomendio & Roldan (1991) who found that sperm lengths were greater in primate and rodent species that generated high risks of sperm competition. Alternatively, Harcourt (1991) also found no relationship between mating pattern and sperm length across primates. Recently, Anderson & Dixson (2002) showed that midpiece volume was greater in primates generating higher risks of sperm competition; competition level was measured through number of sexual partners and relative testis size. We have explored relationships between sperm competition and both mid-piece volume and the volume occupied by the mitochondria across several mammalian orders, but find no evidence for any such relationships. It is possible that when we explore across the wider Mammalia, relationships become confounded by inter-taxonomic variance in other reproductive traits. However, we do control rigorously for phylogeny in our analyses.

When species are used as independent data points within analyses, there is evidence for positive associations between sperm competition level and total sperm length, and the sizes of sperm head, midpiece and flagellar components (table 1*a*). However, species-level comparative analyses are not necessarily instructive for determining evolutionary relationships. We therefore specifically tested for phylogenetic dependence of our data owing to the significant relationships revealed at the species level. Table 1*b* shows that all sperm traits were heavily dependent on phylogeny (as compared with independent, Brownian motion, evolutionary pathways) with maximum lambda values of 1.0. In addition to phylogenetic dependence, this comparative dataset also presents its own specific reasons for requiring phylogenetic control (see electronic Appendix A, available on The Royal Society's Publications Web site). The data are not derived uniformly across the mammals; for example, there is relative over-representation by primate taxa because this order is particularly well studied, while there is only one Marsupial species in the dataset. There is also non-random representation among lower phylogenetic levels; for example, the genus *Pseudomys* has data from eight different species while 53 out of the 83 species are represented by only one species in its genus. Although the dataset is relatively large for this kind of study, this non-uniform representation across taxa generates non-random phylogenetic influences within regressions that analyse at the species level. Thus, combined with the lambda values proving phylogenetic dependence, our analyses after controlling for phylogeny (table 1*b*) therefore describe the unconfounded evolutionary relationships between sperm morphometry and relative testis size.

Testes mass shows typical negative allometry across our 83 mammalian species with a slope exponent of 0.7, which concurs with the value of 0.72 found by Kenagy & Trom-
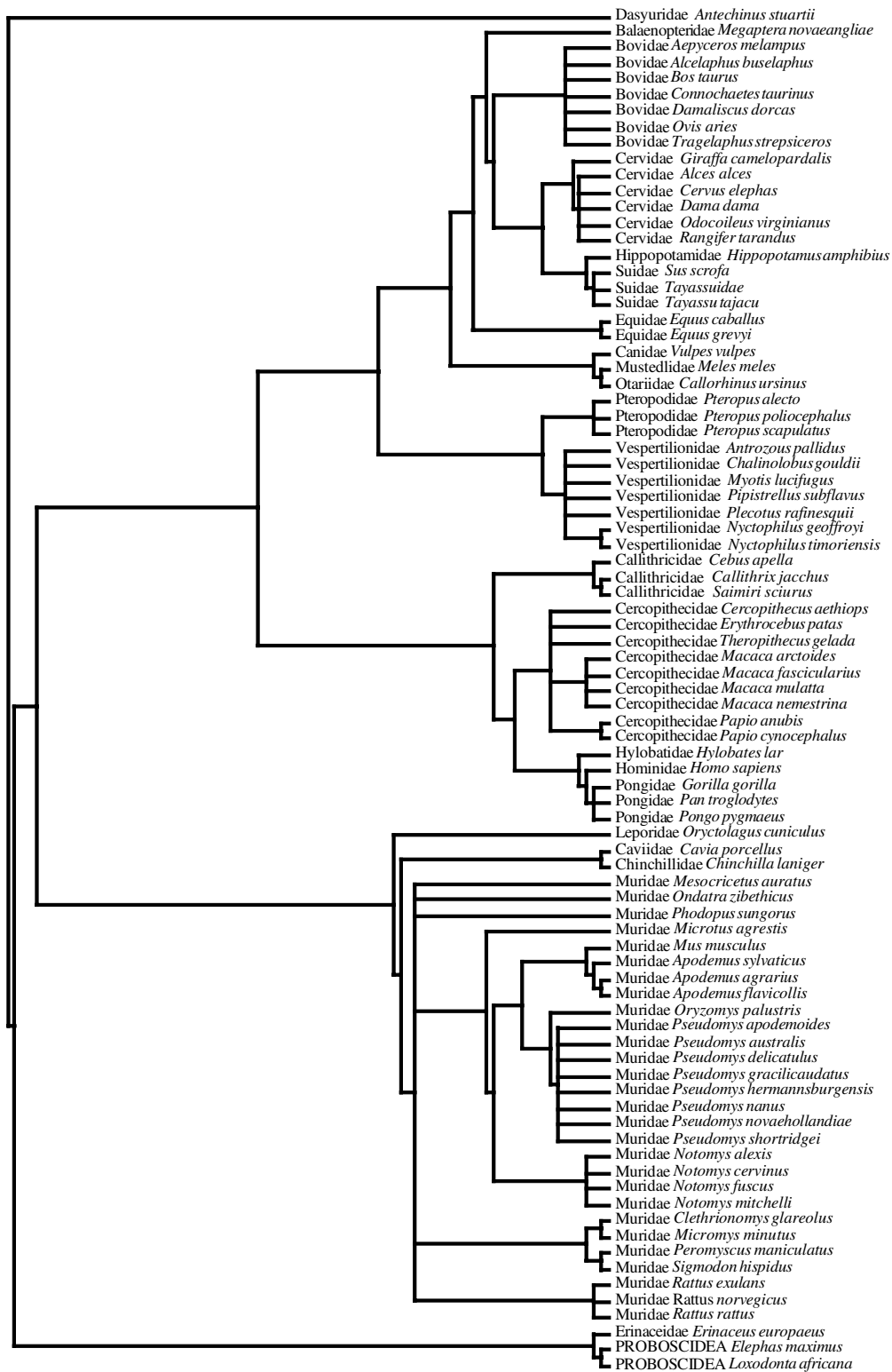
Figure 1. The mammalian phylogeny composed by Liu *et al.* (2001) used in analyses.

bulak (1986) across 133 mammalian species. Such exponents are typical for organ allometry (Harvey & Pagel 1991) and the positive relationship between testis size and body size may result from both humoral and spermatogenic demands on testes. Bigger bodies require greater volumes of hormone to maintain titre, and an increased volume of spermatogenic tissue may be owing to selection from larger female reproductive tracts (Harcourt *et al.* 1981).

By using relative testes mass as a general measure of mating pattern and sperm competition intensity, this study shows no evolutionary relationship between sperm competition and sperm length. Studies of other taxa have shown positive relationships between sperm competition and sperm size. Across butterflies (Gage 1994) and moths (Morrow & Gage 2000), the length of the fertilizing eupyrene sperm type increases with either spermatophore count (= polyandry) or relative testis size across taxa. Addition-
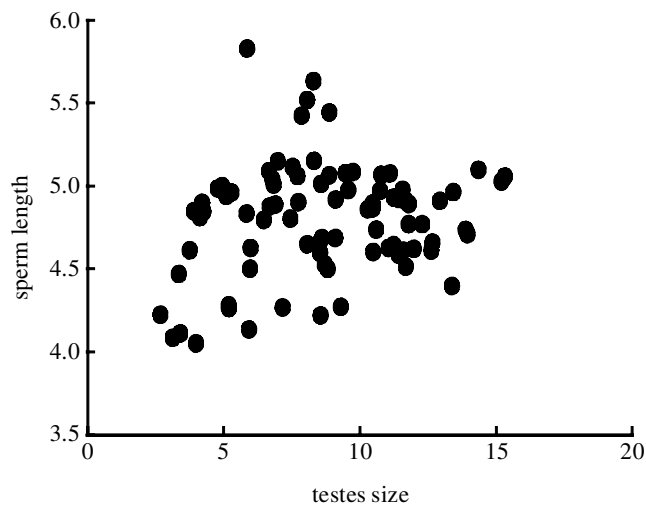
Figure 2. The relationship between testes size and sperm length. In this graph sperm length has been corrected for variations in body size among species, thus the graph shows the effect of testes size controlling for differences in body size between species.

ally, in birds (Briskie & Montgomerie 1992; Johnson & Briskie 1999) and cichlid fishes (Balshine *et al.* 2001) there are also positive relationships between increased sperm competition and greater sperm lengths. The precise mechanism of sperm competition and how sperm form relates to function will explain these relationships. Interestingly, sperm length also relates to female tract dimensions in lepidopterans (Morrow & Gage 2000) and birds (Briskie & Montgomerie 1992) indicating coevolution between mating pattern, female tract dimensions and sperm sizes.

Several other forces may explain the variation in sperm morphometry recognized across mammals (Gage 1998). Other comparative studies have demonstrated a relationship between female tract dimensions and sperm size (Dybas & Dybas 1981; Briskie & Montgomerie 1992; Pitnick *et al.* 1995; Presgraves *et al.* 1999; Morrow & Gage 2000). Across mammals, female tract dimensions are likely to scale with body mass (Harcourt *et al.* 1981); however, no relationship has been found, to our knowledge, between body mass and sperm morphometry in mammals (Gage 1998) despite analyses across 300 species with a range of body sizes that spanned 4 to $>30 \times 10^6$ g (and therefore varied by a factor of over 7 million). In mammals, mechanisms of sperm transport after ejaculation are poorly understood and the female's active role in sperm migration is controversial (Overstreet & Katz 1990). However, details within these mechanisms, and/or the dimensions of specific micro-environments (such as within the cervix or oviduct) within the female reproductive tract may influence sperm morphometry.

The longevity demands on spermatozoa are likely to vary between species depending upon female oestrous patterns, and this could translate into variance in sperm length if size influences longevity (Gomendio & Roldan 1993; Stockley *et al.* 1997). Parker (1984) first identified a positive association between oestrous duration and sperm fertile lifespan and this finding has been further examined by Gomendio & Roldan (1993). However, across 65 mammalian species, Gage (1998) found no evidence for

any relationship between sperm morphometry and the wide variation in oestrus duration (from 2 to more than 5000 hours). It is possible, however, that variance between species in the degree of active female glycolytic support of spermatozoa may confound relationships between sperm longevity and demands on lifespan (Cardullo & Baltz 1991).

The 12-fold variation in described sperm sizes across mammals therefore remains largely unexplained. Further alternatives shaping sperm form and function include the potential for karyotype to influence sperm architecture, although there was no evidence across mammals for relationships between chromosome number or genome mass and sperm morphometry (Gage 1998). Pleiotropic effects from female gamete optima could affect sperm form via linkage disequilibrium, but there was no evidence for egg-size relationships across birds (Johnson & Briskie 1999). Egg vestment traits could influence sperm form and function via selection on spermatozoal ability to penetrate the ovum but there was no relationship between zona pellucida thickness and sperm morphometry across 15 mammalian species (Gomendio & Roldan 1993), and longer sperm were not associated with larger egg diameter across fishes (Stockley *et al.* 1996). It seems probable that both male- and female-derived influences affect the evolution of sperm morphometry. Experimentally controlled variance in sperm morphometry (perhaps by selective breeding), combined with experiments in environments to which sperm are naturally adapted, will enable understanding to progress beyond comparative and theoretical investigations.

REFERENCES

Anderson, M. J. & Dixson, A. F. 2002 Motility and the mid-piece in primates. *Nature* **416**, 496.

Balshine, S., Leach, B. J., Neat, F., Werner, N. Y. & Montgomerie, R. 2001 Sperm size of African cichlids in relation to sperm competition. *Behav. Ecol.* **12**, 726–731.

Birkhead, T. R. & Møller, A. P. 1992 *Sperm competition in birds: evolutionary causes and consequences.* London: Academic.

Birkhead, T. R. & Møller, A. P. 1998 *Sperm competition and sexual selection.* London: Academic.

Birkhead, T. R., Martinez, J. G., Burke, T. & Froman, D. P. 1999 Sperm mobility determines the outcome of sperm competition in the domestic fowl. *Proc. R. Soc. Lond.* B **266**, 1759–1764. (DOI 10.1098/rspb.1999.0843.)

Briskie, J. V. & Montgomerie, R. 1992 Sperm size and sperm competition in birds. *Proc. R. Soc. Lond.* B **247**, 89–95.

Cardullo, R. A. & Baltz, J. M. 1991 Metabolic regulation in mammalian sperm: mitochondrial volume determines sperm length and flagellar beat frequency. *Cell Motil. Cytoskel.* **19**, 180–188.

Darwin, C. 1871 *The descent of man.* New York: Prometheus Books.

Dixson, A. F. 1993 Sexual selection, sperm competition and the evolution of sperm length. *Folia Primatol.* **61**, 221–227.

Dixson, A. F. 1998 *Primate sexuality: comparative studies of the prosimians, monkeys, apes, and human beings.* Oxford University Press.

Dobson, A. J. 1990 *An introduction to generalized linear models.* London: Chapman & Hall.

Dybas, L. K. & Dybas, H. S. 1981 Coadaptation and taxonomic differentiation of sperm and spermathecae in featherwing beetles. *Evolution* **35**, 168–174.

Felsenstein, J. 1973 Maximum likelihood estimation of evolutionary trees from continuous characters. *Am. J. Hum. Genet.* **25**, 471–492.

Felsenstein, J. 1985 Phylogenies and quantitative methods. *A. Rev. Ecol. Syst.* **19**, 445–471.

Freckleton, R. P. 2000 Phylogenetic tests of ecological and evolutionary hypotheses: checking for phylogenetic independence. *Funct. Ecol.* **14**, 129–134.

Freckleton, R. P. 2002 On the misuse of residuals in ecology: regression of residuals versus multiple regression. *J. Anim. Ecol.* **71**, 542–545.

Freckleton, R. P., Harvey, P. H. & Pagel, M. 2002 Phylogenetic analysis and comparative data: a test and review of evidence. *Am. Nat.* **160**, 712–726.

Gage, M. J. G. 1994 Associations between body size, mating pattern, testis size and sperm lengths across butterflies. *Proc. R. Soc. Lond.* B **258**, 247–254.

Gage, M. J. G. 1995 Continuous variation in reproductive strategy as an adaptive response to population density in the moth *Plodia interpunctella*. *Proc. R. Soc. Lond.* B **261**, 25–30.

Gage, M. J. G. 1998 Mammalian sperm morphometry. *Proc. R. Soc. Lond.* B **265**, 97–103. (DOI 10.1098/rspb.1998.0269.)

Gage, M. J. G., Stockley, P. & Parker, G. A. 1995 Effects of alternative male mating strategies on characteristics of sperm production in the Atlantic salmon (*Salmo salar*): theoretical and empirical investigations. *Phil. Trans. R. Soc. Lond.* B **350**, 391–399.

Ginsberg, J. R. & Rubenstein, D. I. 1990 Sperm competition and variation in zebra mating behaviour. *Behav. Ecol. Sociobiol.* **26**, 427–434.

Gomendio, M. & Roldan, E. R. S. 1991 Sperm size and sperm competition in mammals. *Proc. R. Soc. Lond.* B **243**, 181–185.

Gomendio, M. & Roldan, E. R. S. 1993 Coevolution between male ejaculates and female reproductive biology in eutherian mammals. *Proc. R. Soc. Lond.* B **252**, 7–12.

Hansen, T. F. & Martins, E. P. 1996 Translating between microevolutionary process and macroevolutionary patterns: the correlation structure of interspecific data. *Evolution* **50**, 1404–1417.

Harcourt, A. H. 1991 Sperm competition and the evolution of nonfertilizing sperm in mammals. *Evolution* **45**, 314–328.

Harcourt, A. H., Harvey, P. H., Larson, S. G. & Short, R. V. 1981 Testis weight, body weight and breeding system in primates. *Nature* **293**, 55–57.

Harvey, P. H. 1982 On re-thinking allometry. *J. Theor. Biol.* **95**, 37–41.

Harvey, P. H. & Pagel, M. D. 1991 *The comparative method in evolutionary biology.* Oxford University Press.

Heske, E. J. & Ostfeld, R. S. 1990 Sexual dimorphism in size, relative size of testes, and mating systems in North American voles. *J. Mamm.* **71**, 510–519.

Hosken, D. J. 1997 Sperm competition in bats. *Proc. R. Soc. Lond.* B **264**, 385–392. (DOI 10.1098/rspb.1997.0055.)

Hosken, D. J. 1998 Testes mass in megachiropteran bats varies in accordance with sperm competition theory. *Behav. Ecol. Sociobiol.* **44**, 169–177.

Hosken, D. J. & Ward, P. I. 2001 Experimental evidence for testis size evolution via sperm competition. *Ecol. Lett.* **4**, 10–13.

Hutchings, J. A. & Myers, R. A. 1988 Mating success of alternative maturation phenotypes in male Atlantic salmon, *Salmo salar*. *Oecologia* **75**, 169–174.

Jennions, M. D. & Passmore, N. I. 1993 Sperm competition in frogs: testis size and a 'sterile male' experiment on *Chiromantis xerampelina* (Rhacophoridae). *Biol. J. Linn. Soc.* **50**, 211–220.

Johnson, D. D. P. & Briskie, J. V. 1999 Sperm competition and sperm length in shorebirds. *Condor* **101**, 848–854.

Jones, J. W. 1959 *The salmon.* London: New Naturalist.

Katz, D. F. & Drobnis, E. Z. 1990 Analysis and interpretation of the forces generated by spermatozoa. In *Fertilization in mammals* (ed. B. D. Bavister, J. Cummins & E. R. S. Roldan), pp. 125–137. Norwell, MA: Serono Symposia.

Katz, D. F., Drobnis, E. Z. & Overstreet, J. W. 1989 Factors regulating mammalian sperm migration through the female reproductive tract and oocyte vestments. *Gamete Res.* **22**, 443–469.

Kenagy, G. J. & Trombulak, S. C. 1986 Size and function of mammalian testes in relation to body size. *J. Mamm.* **67**, 1–22.

LaMunyon, C. & Ward, S. 1998 Larger sperm outcompete smaller sperm in the nematode *Caenorhabditis elegans*. *Proc. R. Soc. Lond.* B **265**, 1997–2002. (DOI 10.1098/rspb.1998.0531.)

Levitan, D. R. 2000 Sperm velocity and longevity trade off each other and influence fertilization in the sea urchin *Lytechinus variegatus*. *Proc. R. Soc. Lond.* B **267**, 531–534. (DOI 10.1098/rspb.2000.1032.)

Liu, F. G. R., Miyamoto, M. M., Freire, N. P., Ong, P. Q., Tennant, M. R., Young, T. S. & Gugel, K. F. 2001 Molecular and morphological supertrees for eutherian (placental) mammals. *Science* **291**, 1786–1789.

Martin, P. A., Reimers, T. J., Lodge, J. R. & Dzuk, P. J. 1974 The effect of ratios and numbers of spermatozoa mixed from two males on proportions of offspring. *J. Reprod. Fertil.* **39**, 251–258.

Martins, E. P. & Hansen, T. F. 1997 Phylogenies and the comparative method: a general approach to incorporating phylogenetic information into the analysis of interspecific data. *Am. Nat.* **149**, 646–667.

Møller, A. P. 1988*a* Ejaculate quality, testes size and sperm production in primates. *J. Hum. Evol.* **17**, 479–488.

Møller, A. P. 1988*b* Testis size, ejaculate quality and sperm competition in birds. *Biol. J. Linn. Soc.* **33**, 273–283.

Møller, A. P. & Briskie, J. V. 1995 Extra-pair paternity, sperm competition and the evolution of testis size in birds. *Behav. Ecol. Sociobiol.* **36**, 357–365.

Morrow, E. H. & Gage, M. J. G. 2000 The evolution of sperm length in moths. *Proc. R. Soc. Lond.* B **267**, 307–313. (DOI 10.1098/rspb.2000.1001.)

Morrow, E. H. & Gage, M. J. G. 2001 Consistent significant variation between individual males in spermatozoal morphometry. *J. Zool.* **254**, 147–153.

Overstreet, J. W. & Katz, D. F. 1990 Interaction between the female reproductive tract and spermatozoa. In *Controls of sperm motility: biological and clinical aspects* (ed. C. Gagnon), pp. 63–76. Boca Raton, FL: CRC Press.

Pagel, M. 1997 Seeking the evolutionary regression coefficient: an analysis of what comparative methods measure. *J. Theor. Biol.* **164**, 191–205.

Pagel, M. 1999 Inferring the biological patterns of evolution. *Nature* **401**, 877–884.

Parker, G. A. 1970 Sperm competition and its evolutionary consequences in the insects. *Biol. Rev.* **45**, 525–567.

Parker, G. A. 1982 Why are there so many tiny sperm? Sperm competition and the maintenance of two sexes. *J. Theor. Biol.* **96**, 281–294.

Parker, G. A. 1984 Sperm competition and the evolution of animal mating systems. In *Sperm competition and the evolution of animal mating systems* (ed. R. L. Smith), pp. 1–60. Orlando, FL: Academic.

Parker, G. A., Ball, M. A., Stockley, P. & Gage, M. J. G. 1997 Sperm competition games: a prospective analysis of risk assessment. *Proc. R. Soc. Lond.* B **264,** 1793–1802. (DOI 10.1098/rspb.1997.0249.)

Petersen, C. W. & Warner, R. R. 1998 Sperm competition in fishes. In *Sperm competition and sexual selection* (ed. T. R. Birkhead & A. P. Møller), pp. 435–463. London: Academic.

Pitnick, S., Markow, T. & Spicer, G. S. 1995 Delayed male maturity is a cost of producing large sperm in *Drosophila*. *Proc. Natl Acad. Sci. USA* **92,** 10 614–10 618.

Presgraves, D. C., Baker, R. H. & Wilkinson, G. S. 1999 Coevolution of sperm and female reproductive tract morphology in stalk-eyed flies. *Proc. R. Soc. Lond.* B **266,** 1041–1047. (DOI 10.1098/rspb.1999.0741.)

Radwan, J. 1996 Intraspecific variation in sperm competition success in the bulb mite: a role for sperm size. *Proc. R. Soc. Lond.* B **263**, 855–859.

Short, R. V. 1979 Sexual selection and component parts, somatic and genital selection, as illustrated by man and the great apes. *Adv. Study Behav.* **9**, 131–158.

Short, R. V. 1997 The testis: the witness of the mating system, the site of mutation and the engine of desire. *Acta Paediatrica* **86**, 3–7.

Simmons, L. W. 1987 Sperm competition as a mechanism of female choice in the field cricket, *Gryllus bimaculatus*. *Behav. Ecol. Sociobiol.* **21,** 197–202.

Smith, R. L. 1984 *Sperm competition and the evolution of animal mating systems*. London: Academic.

Stockley, P., Gage, M. J. G., Parker, G. A. & Møller, A. P. 1996 Female reproductive biology and the coevolution of ejaculate characteristics in fish. *Proc. R. Soc. Lond.* B **263**, 451–458.

Stockley, P., Gage, M. J. G., Parker, G. A. & Møller, A. P. 1997 Sperm competition in fish: the evolution of testis size and ejaculate characteristics. *Am. Nat.* **149,** 933–954.

Ward, P. I. 1998 Intraspecific variation in sperm size characters. *Heredity* **80**, 655–659.

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